

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :

C07K 15/00

A1

(11) International Publication Number:

WO 94/11403

(43) International Publication Date:

26 May 1994 (26.05.94)

(21) International Application Number: PCT/SE93/00960

(22) International Filing Date: 11 November 1993 (11.11.93)

(30) Priority data:  
9203435-4

11 November 1992 (11.11.92) SE

(71)(72) Applicant and Inventor: MOSBACH, Klaus [SE/SE];  
Lackalänga 31, S-244 94 Furulund (SE).

(72) Inventors; and

(75) Inventors/Applicants (for US only) : VLATAKIS, Georg  
[GR/GR]; Foundation for Research & Technology, Inst.  
of Molecular Biology and Biotech., GR-711 70 Herakli-  
on, Kreta (GR). ANDERSON, Lars, I. [SE/SE]; Tilläm-  
pad Biokemi, P.O. Box 124, S-221 00 Lund (SE). MÜLL-  
ER, Ralf [DE/DE]; Johnson & Johnson, Oststrasse 1,  
D-2000 Norderstedt (DE).(74) Agent: AWAPATENT AB; P.O. Box 5117, S-200 71 Mal-  
mö (SE).(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH,  
CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK,  
LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO,  
RU, SD, SE, SK, UA, US, UZ, VN, European patent  
(AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU,  
MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI,  
CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

(54) Title: ARTIFICIAL ANTIBODIES, METHOD OF PRODUCING THE SAME AND USE THEREOF

## (57) Abstract

Artificial antibodies or antibody mimics are described. They consist of polymers that carry specific binding sites mimicking the properties of antibodies. There is also described a method for producing artificial antibodies, in which polymerisable monomers carrying functional groups and crosslinking monomers are polymerised in the presence of a print molecule and subsequently the print molecule is removed leaving specific binding sites complementary to the print molecules. There are also described methods for determination and isolation of organic molecules using the artificial antibodies as well as therapeutic and diagnostic methods using these antibodies.

BEST AVAILABLE COPY

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TC	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

ARTIFICIAL ANTIBODIES, METHOD OF PRODUCING THE SAME AND  
USE THEREOF

5       The present invention concerns artificial antibodies, a method for producing the artificial antibodies, a method for determination of an organic molecule in a fluid sample, a method for separation or isolation of an organic molecule and use of the latter methods in immunoassays as well as a method of therapy or diagnostics.

10       Antibodies are used in several areas, such as therapy, immunoaffinity, purification and in particular in immunoassays. As to the latter aspect the corresponding antigens can either be small or large molecules.

15       Antibodies are normally produced by immunising animals with the corresponding antigen leading to polyclonal antibodies, or by using fused cells (B cells) allowing the obtained cell lines to produce monoclonal antibodies.

20       Recent efforts in obtaining other biologically derived antibodies or at least antibody-like compounds involve recombinant techniques applied to bacteria or plants.

25       Antibodies can be raised against most compounds; they are versatile reagents employed in numerous applications<sup>1-5</sup>, ranging from basic research to clinical analysis. However, being bio-macromolecules they require careful handling and their production is costly<sup>5</sup>.

30       A potentially useful alternative would be the production of non-biologically derived antibody mimics or artificial antibodies, such as polymer structures that are similar to biological antibodies in binding and recognising antigens.

35       The inherent advantages of such systems would be that the need for animal sources is obliterated, and that antibody mimics can be obtained for cases where it is difficult or impossible to raise antibodies, as for immunosuppressive agents, such as cyclosporin, certain structures, such as macrolides or short peptides.

Furthermore, such non-biological systems could be made more stable, allowing repeated use, higher temperatures and easy sterilisation.

In addition the need for derivatisation of antigens for immunisation purposes is made unnecessary, thereby avoiding the often complicated chemistry and sometimes decreased recognition for the original target molecule (= antigen).

Since the development of the first radioimmunoassay<sup>1</sup>, immunological techniques using labelled reactants have gained an extraordinary prominence in the field of medical research and in clinical diagnosis. In particular, the discovery of monoclonal antibodies<sup>2</sup> and their use in immunoassays has offered novel advantages and more possibilities. Despite the plethora of markers and different procedures<sup>3,4</sup> that have been employed, all the immunological techniques exploit the remarkable affinity and specificity of antibodies. However, antibodies are labile biomolecules which require careful handling and storage. Their production is a time-consuming procedure<sup>5</sup>, including several laborious steps like conjugation of the hapten to a carrier protein, immunisation of animals and isolation of immunoglobulins.

Thus, there was a need for an immunoassay-like technique in which stable and easily prepared highly selective polymers, rather than antibodies are used.

The technique of molecular imprinting has attracted much attention in the last few years<sup>6-8</sup>. Recently, molecular imprinting has been developed to a stage of practical application in enantiomeric separations<sup>11-15</sup>, in particular in the resolution of racemic drugs such as  $\beta$ -blockers<sup>16</sup>.

Furthermore, the technique has been applied to make synthetic enzymes<sup>9,10</sup>.

The technique of molecular imprinting and its special form of non-covalent imprinting as developed by the inventors makes it possible to achieve the above objects.

Briefly, the technique involves polymerisation of functional monomers in the presence of a print molecule (see Scheme 1). Subsequent removal of the print molecule from the rigid polymer results in sites within the polymer  
5 that are complementary to and have an affinity for the original print molecule.

According to the invention there are provided artificial antibodies, which consist of polymers that carry specific binding sites mimicking the properties of anti-  
10 bodies.

There is also provided, according to another aspect of the invention, a method for producing artificial antibodies, in which polymerisable monomers carrying functional groups and crosslinking monomers are polymerised in  
15 the presence of a print molecule and subsequently the print molecule is removed leaving specific binding sites complementary to the print molecule.

The invention also provides for a method for determination of an organic molecule in a fluid sample. According  
20 to this method, a known amount of the organic molecule provided with a label is added to the sample, the sample is contacted with artificial antibodies having specific binding sites for the organic molecule, whereby the labelled and unlabelled organic molecules are competi-  
25 tively bound to the binding sites, and the labelled organic molecule is determined either unbound in the supernatant or bound by the polymer.

There is also provided a method for separation or isolation of an organic molecule from a fluid sample, in  
30 which the sample, labelled or not, is contacted with an excess of artificial antibodies consisting of a polymer having specific sites for the organic molecule, whereby the organic molecule is bound to the binding sites, and optionally the organic molecule is measured bound to the  
35 artificial antibodies or eluted from the antibodies.

The invention also provides for a method of therapy or diagnosis, in which artificial antibodies are administered to a mammal body, which artificial antibodies consist of a biocompatible polymer carrying specific binding sites  
5 mimicking the properties of antibodies towards an organic molecule.

In one embodiment of the invention, the polymers are prepared by non-covalent polymerisation.

The polymers constituting the artificial antibodies  
10 are preferably built up of polymerisable monomers carrying functional groups and crosslinking monomers. Preferably the polymerisable monomers carrying functional groups are chosen among negatively charged monomers such as methacrylic acid, itaconic acid, basic monomers such as vinylpyridine, vinylimidazole, hydrophobic monomers carrying alkyl  
15 chains, monomers allowing  $\pi$ - $\pi$ -interactions, van der Waals forces.

In one embodiment of the invention, polymers are built up of methacrylic acid crosslinked by ethylene  
20 glycol dimethacrylate.

If the artificial antibodies are to be used for administration to a mammal body the polymers must be biocompatible. Preferably they must be of the size not more than 5  $\mu$ m or the size of normal biological antibodies,  
25 most preferred 10-100 nm.

In preparation of artificial antibodies according to the invention, the polymer is ground to a particle size of normally ~ 25  $\mu$ m for use in so-called heterogenous assays.

The fines, that is particles with a size of 10-100 or  
30 1000 nm, resulting from the grinding, can be kept in solution or suspension and used for instance in so-called homogenous immunoassays. Such assays are extremely sensitive and can be performed involving e.g. two different antibodies.

35 Another advantage with the fine particles is that they are more suitable for use in therapy or diagnostics.

Preferably the binding sites are specific for a compound chosen from the group consisting of drugs, metabolites, nucleotides, nucleic acids, carbohydrates, proteins, hormones, toxins, steroids, prostaglandins and leukotrienes.

In one embodiment the binding sites are specific for theophylline or diazepam.

Suitable labels for use in the methods according to the invention are radioligands, enzymes, biotin, steroids, fluorochromes, gold.

The methods according to the invention are preferably used in immunoassays, especially in radioimmunoassays.

The method of therapy or diagnosis according to the invention comprises several different modes of action. For example, it can be used to withdraw an undesired organic molecule from a mammal body, such as a toxin. In another embodiment the artificial antibodies assemble around a cancer cell to indicate the presence of such a cell. In a further embodiment the artificial antibodies are bringing a drug to specific targets, for instance cancer cells.

In one embodiment of treating a mammal body an extra corporal device containing the artificial antibodies is coupled to the body via a shunt in the bloodstream, and the bloodstream is passed through the device.

For the studies the inventors chose two chemically unrelated drugs, theophylline and diazepam, as print molecules. Theophylline, a commonly used drug in the prevention and treatment of asthma, apnea and obstructive lung diseases, has a narrow therapeutic index (56-112  $\mu\text{mol L}^{-1}$  serum) requiring careful monitoring of serum concentrations<sup>17</sup>. Diazepam (e.g. valium) is a member of the benzodiazepine group of drugs widely used as hypnotics, tranquilizers and muscle relaxants<sup>18</sup>. Benzodiazepines are one of the most commonly implicated substances in drug overdose situations and their detection in body fluids is very useful in clinical and forensic toxicology. Current methods for measuring theophylline and benzodiazepines are

based on high-performance liquid chromatography (HPLC)<sup>19-21</sup> and on immunological techniques<sup>22-26</sup>.

The polymers were prepared using methacrylic acid (MAA) as the functional monomer and ethylene glycol dimethacrylate (EDMA) as the crosslinking monomer (Scheme 1). This is a well characterised polymer system that has been used for the preparation of molecular imprints against a number of compounds<sup>12-14,16</sup>. The carboxylic acid function of MAA has been shown to form ionic interactions with amino groups<sup>12</sup> and hydrogen bonds with polar functionalities of the print molecule<sup>14</sup>. The inventors assume that hydrogen bonding is the predominant type of force operating during imprinting and subsequent recognition in the present system. Dipole-dipole and hydrophobic interactions may also contribute.

The solvent compositions giving optimal binding and selectivity were determined for each polymer (see Example 2 and Fig. 1 below). As a general guide<sup>14,27</sup>: i) in a more apolar solvent the substrate binds more strongly to the polymer than in polar solvents, and ii) small amounts of acetic acid can be added to the solvent in order to suppress non-specific binding. The equilibrium dissociation constants ( $K_D$ ) for binding of the drugs to the corresponding polymers were estimated by Scatchard plot analysis using radio-labelled ligands. In both cases, the Scatchard plots were nonlinear and fitted well with two  $K_D$  values, for high and low affinity binding sites. The inventors believe that, as in the case of polyclonal antibodies, the polymers contain a heterogenous population of sites with different affinities for the print molecule. The  $K_D$  values for the high and low affinity binding sites, calculated with the LIGAND programme (Elsevier-Biosoft), were  $3.46 \times 10^{-7}$  M and  $6.55 \times 10^{-5}$  M (associated with a population of sites of  $0.016 \mu\text{mol g}^{-1}$  and  $1.28 \mu\text{mol g}^{-1}$ , respectively) for theophylline and  $3.76 \times 10^{-8}$  M and  $7.36 \times 10^{-8}$  M ( $0.0071 \mu\text{mol g}^{-1}$  and  $0.51 \mu\text{mol g}^{-1}$ ) for diazepam.



Polymers prepared against theophylline or diazepam were used as antibody-substitutes in the construction of competitive binding for theophylline and diazepam determination in human serum. The method, which we name Molecu-  
5 larly Imprinted Sorbent Assay (MIA), relies on the inhibition of binding of radio-labelled ligand by the serum analyte. The amount of radioligand bound to the polymer is inversely related to the concentration of drugs present in the sample. Drug free serum samples spiked with known  
10 amounts of theophylline or diazepam were used for establishing the standard calibration curves. Prior to the actual assay, the drug was extracted from the serum by standard protocols used for HPLC-analysis<sup>19-21</sup> (Fig. 1). The MIA for theophylline was linear over the range  
15 14-224  $\mu\text{mol L}^{-1}$  which is satisfactory for therapeutic monitoring of the drug. The results for diazepam were linear over the range which is normally used in standard immunoassay techniques for benzodiazepines  
(0.44-28  $\mu\text{mol L}^{-1}$ ).  
20 The specificity of the method was tested by the determination of cross-reactivity of major metabolites and of drugs structurally related to theophylline or diazepam (Table 1).

25

30

35

TABLE 1 Cross-reactivity of various xanthine and uric acid derivatives for binding of <sup>3</sup>H-theophylline (bronchodilator) and various benzodiazepines for binding of <sup>3</sup>H-diazepam (tranquilizer) to artificial antibodies (ArtAb's) and natural antibodies (Ab's).

Theophylline antibodies		Diazepam antibodies			
Competitive ligand	Cross-reaction (%)		Competitive ligand	Cross-reaction (%)	
	ArtAb	Ab*		ArtAb	Ab**
Theophylline (1,3-dimethyl-xanthine)					
3-Methylxantin	100	100	Diazepam (e.g. valium)	100	100
Xanthine	7	2	Alprazolam	40	44
Hypoxanthine	<1	<1	Demethyldiazepam	27	32
7-( $\beta$ -Hydroxyethyl)-1,3-dimethylxanthine	<1	<1			
Caffeine (1,3,7-trimethylxanthine)	<1	<1	Clonazepam	9	5
Theobromine (3,7-dimethylxanthine)	<1	<1	Lorazepam	4	1
Uric acid	<1	<1	Chlordiazepoxid	2	<1
1-Methyluric acid	<1	<1			
1,3-Dimethyluric acid	<1	<1			

The ligands were added to drug free serum and assayed as described in Fig. 1. Cross-reactivities are expressed as the molar ratio of theophylline and diazepam, respectively, to ligand giving 50% inhibition of radioligand binding to polymer.

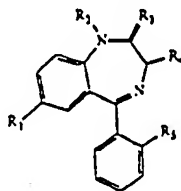
\* Data from ref 22.

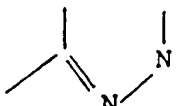
\*\* Data from ref 24.

The MIA method for theophylline (1,3-dimethylxanthine) appears to be highly specific since from all the compounds tested only 3-methylxanthine showed some cross-reactivity.

In the case of the diazepam assay several other  
5 benzodiazepines showed significant cross-reactivity. This was, however, expected because benzodiazepines are very similar in structure, as seen below:

10



		R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
15	Diazepam	Cl	Me	O	H	H
	Desmethyldiazepam	Cl	H	O	H	H
	Clonazepam	NO <sub>2</sub>	H	O	H	Cl
20	Lorazepam	Cl	H	O	OH	Cl
	Alprazolam	Cl			H	H

25

and even antibodies have difficulty in distinguishing between them<sup>25,26</sup> (Table 1).

The ability of the MIA method for accurate measurement of theophylline was evaluated by analysing 32 patient  
30 serum samples. The sample were also analysed with the Enzyme-Multiplied Immunoassay Technique (EMIT)<sup>28</sup> and the comparison of the results obtained showed excellent correlation between the two methods (Fig. 1). Furthermore, the reliability of the assay was determined by measurement of  
35 theophylline samples of known concentration (three clinical significant concentrations; eleven repetitions; coefficient of variation  $\leq 6.5\%$ ).

The results presented here demonstrate, for the first time, the ability to use chemically prepared macromolecules with preselected specificity, instead of the traditional biomolecules, as receptors in competitive binding assays. A great advantage of molecularly imprinted polymers is their simple and rapid (two to three days) preparation and their remarkable stability. They can be stored in the dry state, even at elevated temperatures, for several years without loss of recognition capabilities<sup>27</sup>. In addition, the potential to reuse the polymers may prove valuable. Furthermore, by analogy to immunoaffinity chromatography, molecularly imprinted polymers could be useful for the separation and isolation of different compounds. Apart from the practical importance of the described preparations, structural studies on the interactions of drugs with their artificial receptors could yield valuable insight into the nature of molecular recognition phenomena<sup>29-31</sup>.

Molecular imprints may be obtained against functionality complementary to the monomer<sup>14,27</sup>. There is a potential for molecularly imprinted artificial antibodies in the analysis of many other drugs, metabolites, hormones, toxins, etc.

It is also noteworthy that molecularly imprinted polymers provide a potential alternative to the use of laboratory animals for the production of antibodies. Preliminary data from similar studies with an emphasis on recognition in aqueous systems using other compounds such as opiates and biologically active peptides, indicate that this technique promises to become widely useful.

The invention is described more in detail with reference to the following examples and the accompanying drawing.

Figure 1 shows a comparison of the competitive binding assays Enzyme-Multiplied Immunoassay Technique (EMIT)<sup>28</sup> and MIA for determination of serum concentration of theophylline in patient samples (n=32).

Example 1

## Preparation of molecularly imprinted polymers

The preparation follows the reaction of Scheme 1.

- 5 A) The functional monomer, methacrylic acid (MAA, 1), is mixed with the print molecule, here theophylline (2), and ethylene glycol dimethacrylate (EDMA), the crosslinking monomer, in a suitable solvent. MAA is selected for its ability to form hydrogen bonds with a variety of chemical functionalities of the print molecule.
- 10 B) The polymerisation reaction is started with the addition of initiator (AIBN) and a rigid insoluble polymer is formed. "Imprints", which are complementary in both shape and chemical functionality to the print molecule, are now present within the polymeric network.
- 15 C) The print molecule is removed by extraction.

The wavy lines in Scheme 1 represent an idealised polymer structure but do not take into account the accessibility of the substrate to the recognition site in the macroporous polymer structure.

20 METHODSAnti-theophylline polymer

- To a glass bottle were added chloroform (250 ml), theophylline (4.7 g), MAA (9 g), EDMA (93,5 g) and 2,2'-azobis(2-methylpropionitrile) (AIBN, initiator, 1.2 g).
- 25 The mixture was degassed under vacuum in a sonicating waterbath and sparged with nitrogen for 5 min. The polymerisation reaction took place at 60°C for 24 h. The bulk polymer was grounded in a mechanical mortar and wet sieved (water) through a 25 µm sieve. The fines were removed by
- 30 repeated settling in acetonitrile. The print molecule (theophylline) was extracted by extensive washing of the particles with methanol-acetic acid (9/1, v/v). Finally, the polymer particles were dried under vacuum and stored in a desiccator.

Anti-diazepam polymer

Diazepam (1.27 g) was mixed with MAA (2.26 g), EDMA (26.1 g) and AIBN (0.5 g) in chloroform (39 ml). The polymerisation mixture was degassed under vacuum in a sonicating water-bath, sparged with nitrogen and then polymerised under UV (366 nm) at 4°C for 16 h. The resulting polymer was then treated as described above.

Example 2

A comparison of the competitive binding assays  
10 Enzyme-Multiplied Immunoassay Technique (EMIT)<sup>28</sup> and MIA for determination of serum concentration of theophylline in patient samples (n=32) was performed. EMIT reagents were supplied by the manufacturer (SYVA, Palo Alto, USA). All enzyme immunoassays were preformed at the department  
15 of Clinical Pharmacology, University Hospital, Lund, Sweden, according to the method of the manufacturer. The result is shown in Fig. 1:  
Slope: 0.99, Intercept: 1.50  $\mu\text{mol L}^{-1}$ , correlation coefficient: 0.98.

20 METHODS

The assay conditions were established by applying similar protocols as is standard for the optimisation of immunoassays using antibodies<sup>32</sup>. 40  $\mu\text{l}$  of each sample was mixed with 40  $\mu\text{l}$  of HCl (0.2 M) and extracted with 1 ml of  
25 dichloromethaneisopropanol (4/1, v/v). The organic layer was evaporated at 40°C under a stream of nitrogen. The residue was redissolved in 100  $\mu\text{l}$  of acetonitrile-acetic acid (99/1, v/v) containing [<sup>3</sup>H]-theophylline (5 ng, 18.6 Ci  $\text{mmol}^{-1}$ ). Polymer imprinted against theophylline  
30 was then added (12.5 mg of polymer in 0.9 ml of the same solvent) and the mixture was incubated for 15 h at room temperature. The binding equilibrium was reached after 8 h, 80 and 90% of the binding occurred within 3 and 5 h. After centrifugation, the unbound [<sup>3</sup>H]-theophylline in  
35 200  $\mu\text{l}$  of the supernatant was measured by liquid scintillation counting. The calibration graph was linear over the range 14-224  $\mu\text{mol L}^{-1}$  (correlation coefficient = 0.999)

13

and the detection limit of the assay was found to be  $3.5 \mu\text{mol L}^{-1}$ . The diazepam assay, performed in a similar manner using 5 mg of polymer in toluene-heptane (4:1; v/v), was linear from 0.44 to  $28 \mu\text{mol L}^{-1}$  (correlation coefficient = 0,991) with a detection limit of  $0.2 \mu\text{mol L}^{-1}$ .

10

15

20

25

30

35

References

1. Yalow, R. S. & Berson, S. A. Nature 184, 1648-1649 (1959).
2. Köhler, G. & Milstein, C. Nature 256, 495-497 (1975).
- 5 3. Oellerich, M. J. Clin. Chem. Clin. Biochem. 22, 895-904 (1984).
4. Gosling, J. P. Clin. Chem. 36, 1408-1427 (1990).
5. Kurstak, E. in Enzyme Immunodiagnosis (ed Kurstak, E.) 5-11 (Academic Press, London, 1986).
- 10 6. Ekberg, B. & Mosbach, K. Trends Biotechnol. 7, 92-96 (1989).
7. Wulff, G. Amer. Chem. Soc. Symp. Series 308, 186-230 (1986).
8. Shea, K. J. & Sasaki, D. Y. J. Am. Chem. Soc. 113, 4109-4120 (1991).
- 15 9. Robinson, D. K. and Mosbach, K. J. Chem. Soc. Chem. Commun. 14, 969-970 (1989).
10. US patent No. 5,110,833 to Klaus Mosbach.
11. Sällergren, B., Ekberg, B. & Mosbach, K. J. Chromatogr. 347, 1-10 (1985).
- 20 12. Sällergren, B., Lepistö, M. & Mosbach, K. J. Am. Chem. Soc. 110, 5853-5860 (1988).
13. O'Shannessy, D. J., Ekberg, B., Andersson, L. I. & Mosbach, K. J. Chromatogr. 470, 391-399 (1989).
- 25 14. Andersson, L. I. & Mosbach, K. J. Chromatogr. 516, 313-322 (1990).
15. Wulff, G. & Minarik, M. J. Liq. Chromatogr. 13, 2987-3000 (1990).
16. Fischer, L., Müller, R., Ekberg, B. & Mosbach, K. J. Am. Chem. Soc. 113, 9358-9360 (1991).
- 30 17. Hendeles, L., Weinberger, M. & Johnson, G. Clin. Pharmacokinetics 3, 294-312 (1978).
18. Harvey, S. L. in The Pharmacological Basis of Therapeutics (eds Gilman, A. G., Goodman, L. S., Rail, T. W. & Murad, F.) 339-351 (Marcel Dekker Inc., New York, 1985).
- 35



19. Meffin, P. J. & Miners, J. O. in Progress in Drug Metabolism (eds Bridges, J. W. & Chasseaud, L. F.) Vol. 4, 261-307 (J. Wiley, London, 1980).
20. Peng, G. W., Gadalla, M. A. F. & Chiou, W. L. Clin. Chem. 24, 357-361 (1978).
21. Mura, P., Piriou, A., Fraillon, P., Papet, Y. & Reiss, D. J. Chromatogr. 416, 303-310 (1987).
22. Castro, A., Ibanez, J., Voight, W., Noto, T. & Malkus, H. Clin. Chem. 24, 944-946 (1978).
23. Chang, J., Gotcher, S. & Gushaw, J. B. Clin. Chem. 26, 361-367 (1982).
24. Poncelet, S. M., Limet, J. N., Noel, J. P., Kayaert, M. C., Galanti, L. & Collet-Cassart, D. J. Immunoassay, 11, 77-88 (1990).
25. Baselt, R. C. in Advances in Analytical Toxicology (ed Baselt, R. C.) Vol. 1, 81-123 (Biomedical Publications, Foster City, CA, 1984).
26. Aitunkaya, D. & Smith, R. N. Forensic. Sci. Int., 39, 23-37 (1988).
27. Andersson, L. I. thesis, Lund Univ. (1991).
28. Dietzler, D. N., Waldner, N., Tieber, V. L., McDonald, J. M., Smith, C. H., Ladenson, J. H. & Leckie, M. P. Clin. Chim. Acta 101, 163-181 (1980).
29. Cram, D. J. Nature 356, 29-36 (1992).
30. Rebek, J. Jr. Angew. Chem. Int. Ed. Engl. 29, 245-255 (1990).
31. Desiongchamps, G., Galán, A., de Mendoza, J. & Rebek, J. Jr. Angew. Chem. Int. Ed. Engl. 31, 61-63 (1992).
32. Tijssen, P. Laboratory Techniques in Biochemistry and Molecular Biology, Practice and Theory of Enzyme Immunoassays 5th printing (Elsevier Publishers B.V., Amsterdam, 1988).

16

SCHEME 1

5

10

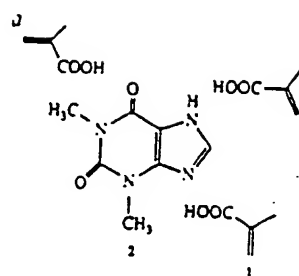
15

20

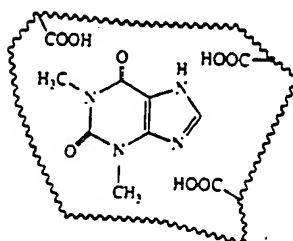
25

30

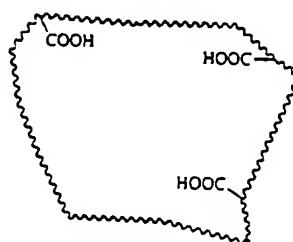
35



b  
polymerization



c  
extraction



## CLAIMS

1. Artificial antibodies, c h a r a c t e r i s e d  
5 in that they consist of polymers that carry specific binding sites mimicking the properties of antibodies.

2. Artificial antibodies according to claim 1,  
c h a r a c t e r i s e d in that the polymers are prepared by polymerisation of polymerisable monomers carrying  
10 functional groups and crosslinking monomers.

3. Artificial antibodies according to claim 1 or 2,  
c h a r a c t e r i s e d in that the polymers are prepared by non-covalent polymerisation.

4. Artificial antibodies according to claim 2 or 3,  
15 c h a r a c t e r i s e d in that the polymerisable monomers carrying functional groups are chosen among negatively charged monomers such as methacrylic acid, itaconic acid, basic monomers such as vinylpyridine, vinylimidazole, hydrophobic monomers carrying alkyl  
20 chains, monomers allowing  $\pi$ - $\pi$ -interactions, van der Waals forces.

5. Artificial antibodies according to any one of the preceding claims, c h a r a c t e r i s e d in that the polymers are built up of methacrylic acid cross-  
25 linked by ethylene glycol dimethacrylate.

6. Artificial antibodies according to any one of the preceding claims, c h a r a c t e r i s e d in that the polymers are biocompatible.

7. Artificial antibodies according to claim 6,  
30 c h a r a c t e r i s e d in that they are of a size of not more than 5  $\mu$ m, preferably 10-100 nm.

8. Artificial antibodies according to any one of the preceding claims, c h a r a c t e r i s e d in that the binding sites are specific for a compound chosen from the  
35 group consisting of drugs, metabolites, nucleotides, nucleic acids, carbohydrates, proteins, hormones, toxins, steroids, prostaglandins and leukotrienes.

9. Artificial antibodies according to any one of the preceding claims, characterised in that the binding sites are specific for theophylline.

10. Artificial antibodies according to any one of claims 1-8, characterised in that the binding sites are specific for diazepam.

11. A method for producing artificial antibodies, characterised in that polymerisable monomers carrying functional groups and crosslinking monomers are polymerised in the presence of a print molecule and subsequently the print molecule is removed, leaving specific binding sites complementary to the print molecules.

12. A method according to claim 11, characterised in that the polymerisation is a non-covalent polymerisation.

13. A method according to claim 11 or 12, characterised in that the polymerisable monomers are chosen among negatively charged monomers such as methacrylic acid, itaconic acid, basic monomers such as vinylpyridine, vinylimidazole, hydrophobic monomers carrying alkyl chains, monomers allowing  $\pi$ - $\pi$ -interactions, van der Waals forces.

14. A method according to any one of claims 11-13, characterised in that the polymerisable monomers are methacrylic acid and the crosslinking monomers are ethylene glycol dimethacrylate.

15. A method according to any one of claims 11-14, characterised in that the polymers are made into a size of not more than 5  $\mu$ m, preferably 10-100 nm.

16. A method according to any one of claims 11-15, characterised in that the print molecule is chosen from the group consisting of drugs, metabolites, nucleotides, nucleic acids, carbohydrates, proteins, hormones, toxins, steroids, prostaglandins and leukotrienes.

17. A method according to any one of claims 11-16, characterised in that the print molecule is theophylline.

18. A method according to any one of claims 11-16, characterised in that the print molecule is diazepam.

19. A method for determination of an organic molecule  
5 in a fluid sample, characterised in that a known amount of the organic molecule provided with a label is added to the sample, the sample is contacted with artificial antibodies as claimed in any one of claims 1-9 having specific binding sites for the organic molecule,  
10 whereby the labelled and unlabelled organic molecules are competitively bound to the binding sites, and the labelled organic molecule is determined either unbound in the supernatant or bound by the polymer.

20. A method according to claim 19, characterised  
15 terised in that the label is chosen from the group consisting of radioligands, enzymes, biotin, steroids, fluorochromes, electrochemiluminescent compounds, gold.

21. Use of the method according to claim 19 or 20 in heterogenous or homogenous immunoassays.

20 22. Use according to claim 21 in homogenous immunoassays, whereby the artificial antibodies are of a size of not more than 5  $\mu\text{m}$ , preferably 10-100 nm.

23. A method for separation or isolation of an organic molecule from a fluid sample, characterised  
25 terised in that the sample, labelled or not, is contacted with an excess of artificial antibodies as claimed in any one of claims 1-9 having specific sites for the organic molecule, whereby the organic molecule is bound to the binding sites, and optionally the organic  
30 molecule is measured bound to the artificial antibodies or eluted from the antibodies.

24. A method of therapy or diagnosis, characterised  
35 terised in administration of artificial antibodies to a mammal body, which artificial antibodies consist of a biocompatible polymer carrying specific binding sites mimicking the properties of antibodies towards an organic molecule.

20

25. A method according to claim 24, c h a r a c -  
t e r i s e d in that an extracorporeal device containing  
the artificial antibodies is coupled to the body via a  
shunt in the bloodstream, and the bloodstream is passed  
5 through the device.

26. A method according to claim 23 or 24, c h a -  
r a c t e r i s e d in that the artificial anitbodies are  
of a size of not more than 5  $\mu\text{m}$ , preferably 10-100 nm.

10

15

20

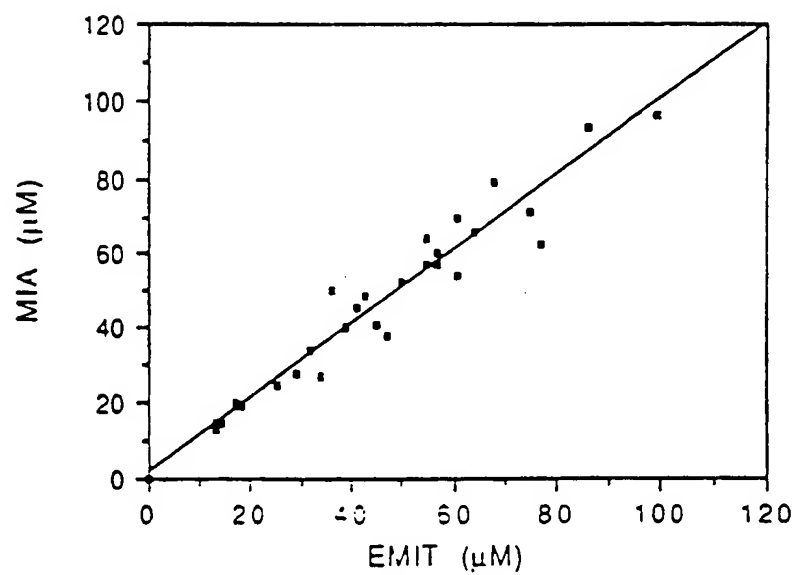
25

30

35

1 / 1

FIG. 1



1  
INTERNATIONAL SEARCH REPORT

International application No.  
PCT/SE 93/00960

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C07K 15/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, WPIL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5110833 (K MOSBACH), 5 May 1992 (05.05.92), column 2, line 18 - line 40; column 3, line 13 - line 46, claims --	1-8, 11-16, 19-23
X	Dialog Information Services, File 34, Scisearch, Dialog accession no. 10998079, Kempe M et al: "Binding-studies on substrate-and enantio-selective molecularly imprinted polymers", Analytical letters, 1991, V24, N7, P1137-1145 --	1-8, 11-16

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

14 February 1994

Date of mailing of the international search report

17 -02- 1994

Name and mailing address of the ISA/  
Swedish Patent Office  
Box 5055, S-102 42 STOCKHOLM  
Facsimile No. +46 8 666 02 86

Authorized officer

Carl Olof Gustafsson  
Telephone No. +46 8 782 25 00



2  
INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 93/00960

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	National Library of Medicine database, File Medline, NLM accession no. 91177967, Andersson LI: "Enantiomeric resolution on molecu- larly imprinted polymers prepared with only non- covalent and non-ionic interactions", J Chromatogr 1990 Sep 21;516(2):313-22  --	1-8,11-16,23
X	Dialog Information Services, File 154, Medline, Dialog accession no. 07658968, Medline accession no. 91177968, Andersson LI et al: "Enatiomeric resolution of amino acid derivatives on molecu- larly imprinted polymers as monitored by potentio- metric measurements", J Chromatorgr Sep 21 1990, 516 (2) p 323-31  --	1,8,11,23
X	National Library of Medicine, File Medline, NLM accession no. 90267842, O'Shannessy DJ et al: "Molecular recognition in synthetic polymers. Enantiomeric resolution of amide derivatives of amino acids on molecularly imprinted polymers", J Mol Recognit 1989 Jul;2(1):1-5  --	1,8,11,23
X	Dialog Information Services, File 351, WPIL, Dialog accession no. 004677980, WPI accession no. 86-181322/28, Mosbach K: "Phenylalanine ethyl ester selective polymer produced by molecular imprinting of rigid crosslinked polymer", SE 8404967 A 860405 8628 (Basic)  --	1,8,11,23
P,X	National Library of Medicine database, File Medline, NLM accession no. 93173199, Vlatakis G: "Drug assay using antibody mimics made by molecular imprinting", Nature 1993 Feb 18;361(6413):645-7  --	1-23

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 93/00960

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>Dialog Information Services, File 34, Scisearch, Dialog accession no. 12613654, Hedborg E et al: "Some studies of molecularly-imprinted polymer membranes in combination with field-effect devices", Sensors and Actuators A-physical, 1993, V37-8, jun- (jun-aug), p796-799</p> <p style="text-align: center;">--- -----</p>	1,8,11

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 93/00960

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 24-26  
because they relate to subject matter not required to be searched by this Authority, namely:  
The wording "artificial antibodies" consist of polymers that carry specific binding sites" is too broad to permit an adequate search.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

28/01/94

International application No.

PCT/SE 93/00960

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 5110833	05/05/92	SE-A- 8900130	16/01/89

**PThis Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☒ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**

**THIS PAGE BLANK (USPTO)**